

Bruce A. Keyt, Ph.D.

Birthdate: November 24, 1952

Address: 612 Rockaway Beach Avenue
Pacifica, California 94044

Education: Doctor of Philosophy (1981)
Tufts University School of Medicine
Sackler School of Graduate Studies
Department of Biochemistry and Pharmacology
Boston, Massachusetts

Bachelors Degree (1973)
Washington University
School of Arts and Sciences
Department of Chemistry
St. Louis, Missouri

Post-Graduate: Molecular Modeling Workshop
BioSym Technologies, Inc.
San Diego, CA.

Pharmacokinetics for Pharmaceutical Scientists
University of California, San Francisco
School of Pharmacy

Employment: Yale University Medical School
111 Congress Avenue
New Haven, Connecticut 06511

1973-1974 Assistant in Research
Physical Sciences Laboratory

1974-1975 Associate in Research
Laboratory of Oncology

Genentech, Inc.
460 Point San Bruno Boulevard
South San Francisco, California 94080

1982-1983 Post-doctoral Fellow
Department of Protein Chemistry

1984-1987 Development Scientist
Department of Medicinal and Analytical Chemistry

1988-1993 Research Scientist
Department of Cardiovascular Research

1993 Project Team Leader for Vascular Endothelial Growth Factor

Professional Societies:

American Association for the Advancement of Science
American Chemical Society
American Heart Association-- Thrombosis Council
Association of Biomolecular Resource Facilities
California Separation Science Society
International Society for Fibrinolysis and Thrombosis
International Society on Thrombosis and Hemostasis
New York Academy of Science
The Protein Society

Book Chapters and Review Articles:

1. Furie, B.C., Borowski, M.A., **Keyt, B.**, Furie, B. (1982) Calcium and Cell Function, vol. II, 217-242, Academic Press, Cheung, W.A., Editor "Gamma-carboxyglutamic acid containing calcium binding proteins"
2. Ling, V. T., Eng, M. L., Lee, P.-J., Keck, R. G., **Keyt, B. A.**, and Canova-Davis, E. Critical Reviews in Chemistry "Nuances of Peptide Mapping in the Analysis of Protein Pharmaceuticals"
3. **Keyt, B. A.**, Paoni, N. F., and Bennett, W. F., "Site-Directed Mutagenesis of Tissue Plasminogen Activator." (manuscript in review) Hanser Publishing, Cleland, J. L. and Craik, C. S. Eds.

Publications:

1. Sperling, R., Furie, B.C., Blumenstein, M., **Keyt, B.**, and Furie, B. (1978) *J. Biol. Chem.* **253**, 3898-3906 "Metal Binding Properties of Gamma-Carboxyglutamic Acid"
2. **Keyt, B.A.**, Furie, B.C., and Furie, B. (1982) *J. Biol. Chem.* **257**, 8687-8695 "Alterations in the antigenic surface of Bovine Factor X accompanying zymogen activation"
3. Wood, W.I., Capon, D.J., Simonsen, C.C., Eaton, D.L., Gitschier, J., **Keyt, B.**, Seeburg, P.H., Smith, D.H., Hollingshead, P., Wion, K., Delwart, E., Tuddenham, E.G.D., Vehar, G.A., and Lawn, R.M. (1984) *Nature* **312**, 330-337 "Expression of active human factor VIII from recombinant DNA clones"
4. Vehar, G.A., **Keyt, B.**, Eaton, D., Rodriguez, H., O'Brien, D.P., Rotblat, F., Oppermann, H., Keck, R., Wood, W.I., Harkins, R.N., Tuddenham, E.G.D., Lawn, R.M., and Capon, D.J. (1984) *Nature* **312**, 337-342 "Structure of human factor VIII"
5. Vehar, G.A., Spellman, M.W., **Keyt, B.A.**, Ferguson, C.K., Keck, R.G., Chloupek, R.C., Harris, R., Bennett, W.F., Builder, S.E., and Hancock, W.S. (1986) *Cold Spring Harbor Symposia on Quantitative Biology* **51**, 551-562 "Characterization Studies of Human Tissue-type Plasminogen Activator Produced by Recombinant DNA Technology"
6. Mohler, M.A., Tate, K., Bringman, T.S., Fuller, G., **Keyt, B.**, Vehar, G., Hotchkiss, A.J. (1988) *Fibrinolysis* **2**, 17-23 "Circulatory Metabolism of Recombinant Tissue-type Plasminogen Activator in Monkeys and Rabbits"

7. Chloupek, R.C., Harris, R.J., Leonard, C.K., Keck, R.G., **Keyt, B.A.**, Spellman, M.W., Jones, A.J.S., Hancock, W.S. (1989) *J. Chromatog.* **463**, 375-396 "Study of the primary structure of recombinant tissue plasminogen activator by reversed-phase high-performance liquid chromatographic tryptic mapping"
8. Anicetti, V.R., **Keyt, B.A.**, and Hancock, W.S. (1989) *Trends in Biotechnol.* **7**, 342-349 "Purity analysis of protein pharmaceuticals produced by recombinant DNA technology"
9. Bennett, W.F., Paoni, N.F., **Keyt, B.A.**, Botstein, D., Jones, A.J.S., Presta, L., Wurm, F., Zoller, M. (1991) *J. Biol. Chem.* **266**, 5191-5201 "High resolution analysis of functional determinants on human tissue-type plasminogen activator"
10. **Keyt, B.A.**, Berleau, L.T., Nguyen, H.V., and Bennett, W.F., (1992) *Analytical Biochemistry* **206**, 73-83 "Radioiodination of the Active Site of Tissue Plasminogen Activator: A Method for Radiolabeling Serine Proteases with Tyrosyl-prolyl-arginyl-Chloromethyl Ketone"
11. Paoni, N.F., **Keyt, B.A.**, Refino, C.J., Chow, A. M., Nguyen, H.V., Berleau, L.T., Badillo, J., Pena, L.C., Brady, K., Wurm, F.M., Ogez, J. and Bennett, W.F. (1993) *Thrombosis and Haemostasis* **7** (2) 307-312 "A Slow Clearing, Fibrin-Specific, PAI-1 Resistant Variant of tPA (T103N, KHRR296-299AAAA)."
12. Refino, C.J., Paoni, N.F., **Keyt, B.A.**, Pater, C.S., Badillo, J.M., Wurm, F. M., van Reis, R., and Bennett, W.F. (1993) *Thrombosis and Haemostasis* **70** (2) 313-319 "A Variant of tPA (T103N, KHRR296-299AAAA) that, by Bolus, Has Increased Potency and Decreased Systemic Activation of Plasminogen."
13. Paoni, N.F., Chow, A.M., Pena, L.C., **Keyt, B.A.**, Zoller, M.J., and Bennett, W.F. (1993) *Protein Engineering* **6** (5) 529-534 "Making Tissue-type Plasminogen Activator More Fibrin Specific"
14. Guzzetta, A.W., Basa, L.J., Hancock, W.S., **Keyt, B.A.**, and Bennett, W.F. (1993) *Analytical Chemistry* **65**, 2953-2962 "Identification of Carbohydrate Structures in Glycoprotein Peptide Maps by the Use of LC/MS with Selected Ion Extraction with Special Reference to Tissue Plasminogen Activator and a Glycosylation Variant Produced by Site Directed Mutagenesis"
15. **Keyt, B. A.**, Paoni, N. F., Refino, C.J., Ogez,J., Etcheverry,T., Botstein, D. and Bennett, W. F., (manuscript submitted to *Proc. Natl. Acad. Sci. USA.*) "A Faster-Acting and More Potent Form of Tissue Plasminogen Activator."
16. Orth, K., Gething, M.-J., **Keyt, B.**, and Sambrook, J., (manuscript submitted to *J. Biol. Chem.*) "The Apparent Rate of Degradation of Tissue-type Plasminogen Activator Depends on the Method of Radiolabeling"
- Collen, D., Stassen, J.M., Refino, C.J., Paoni, N.F., **Keyt, B.A.**, Roskams, T., Lijnen, H.R., and Bennett, W.F. (manuscript in preparation) "Comparative Thrombolytic Properties of Tissue-type Plasminogen Activator and of Two Plasminogen Activator Inhibitor-1 Resistant Glycosylation Variants, in a Combined Arterial and Venous Thrombosis Model in the Dog"

Thomas, G. R., Thibodeaux, H., Errett, C. J.; Badillo, J. M., **Keyt, B. A.**, Paoni, N. F., Refino, C. J., Zivin, J. A., and Bennett, W. F., (manuscript in preparation) "A Long Half-Life, Fibrin Specific, Tissue-Type Plasminogen Activator Reduces Cerebral Bleeding in a Rabbit Model of Embolic Stroke"

Sinicropi, D., Dere, R., Higgins, D., Schrader, B., Mensing, M., **Keyt, B.A.**, Fendly, B., Marks, B., Patzer, E., and Chen, A. (manuscript in preparation) "Quantitation of Recombinant Human Tissue-Type Plasminogen Activator Variants with Monoclonal Antibodies Directed Against Conserved Structural Domains"

Paoni, N.F....**Keyt, B.A.**, etal. "Engineering the Therapeutic Properties of Human tPA" (manuscript in preparation)

Keyt, B.A.....Bennett, W.F., etal. "Localization of the Clearance Determinants on Tissue Plasminogen Activator using Alanine Scan Variants" (manuscript in preparation)

Abstracts:

1. **Keyt, B.**, Furie, B.C., and Furie, B. (1980) *Fed. Proc.* **39**, 1894 "Immunological Characterization of Structural Transitions in Factor X during Zymogen Activation"
2. Vehar, G.A., Eaton, D.L., **Keyt, B.**, Capon, D.J., Wood, W.I., Lawn, R.M., and Tuddenham, E.G.D. (1985) *Thromb. Haemostas.* **54**, 116 "Structure of Human Factor VIII"
3. Chloupek, R.C., **Keyt, B.A.**, Harris, R.J., Keck, Rodney, R.G. and Jones, A.J.S. (1985) Fifth International Symposium on HPLC of Proteins, Peptides, and Polynucleotides, November 4-6, Toronto, Ontario, Canada "Characterization of the Primary Structure of Recombinant Tissue Plasminogen Activator"
4. **Keyt, B.A.**, Harris, R.J., Doherty, T., Keck, R.G., Jones, A.J.S. (1986) *J. Cellular Biochem. Suppl.* 10A, 250 "Electrophoretic and Immunochemical Characterization of Tissue Plasminogen Activator"
5. **Keyt, B.A.**, Teshima, G., Harris, R.J., Jones, A., (1986) HPLC '86, San Francisco, CA., Abstract 813 "Limited Proteolytic Digestion of Tissue Plasminogen Activator"
6. Harris, R.J., Spellman, M.W., and **Keyt, B.A.** (1986) 6th International Conference on Methods in Protein Sequence Analysis, Seattle, WA. Abstract D66 "Kringle region heterogeneity in recombinant tissue-type plasminogen activator is due to carbohydrate differences at Asn-184"
7. Teshima, G., Harris, R., Keck, R., Meunier, A., Burnier, J., and **Keyt, B.** (1987) *Thromb. Haemostas.* **58**, 429 "Characterization of limited proteolytic digests of tissue plasminogen activator"
8. **Keyt, B.A.**, Higgins, D., and Meunier, A. (1988) *Fibrinolysis* **2**, suppl.1, 107 "Conversion of single chain tPA to the two chain form during fibrinolysis"
9. **Keyt, B.**, (1989) 27th Eastern Analytical Symposium, New York, N.Y., Abstract 202 "Reverse Phase HPLC Analysis of Recombinant Tissue Plasminogen Activator"

10. Lee, P.J., Teshima, G., Keck, R., **Keyt, B.**, and Canova-Davis, E. (1989) Third Symposium of the Protein Society, Seattle, WA., Abstract M96 "Identification of a degradation product of recombinant human tissue plasminogen activator produced at low pH"
11. **Keyt, B.**, Nguyen, H., Cachianes, G., and Leung, D. (1989) The 2nd International Workshop on the Molecular and Cellular Biology of Plasminogen Activation, Long Island, N.Y. "Fibrin binding characteristics of tissue plasminogen activator mutants"
12. Sinicropi, D., Dere, R., Higgins, D., Schrader, B., **Keyt, B.**, Fendly, B., Marks, B., Patzer, E., and Chen, A.B. (1989) *Thromb. Haemostas.* **62**, 395 "Generation of Monoclonal Antibodies against Recombinant Human Tissue-type Plasminogen Activator and Determination of the Structural Domains to which they Bind"
13. Chen, S.A., Foster, D.L., **Keyt, B.A.**, Fendly, B., Sinicropi, D., Baughman, R.A., and Billings, R. (1989) *Thromb. Haemostas.* **62**, 337 "Correlation of hepatocyte uptake with *in vivo* clearance of recombinant tissue-type plasminogen activator and identification of the domains responsible for clearance"
14. Hancock, W.S., Canova-Davis, E., Battersby, J., Harris, R., Keck, R., and **Keyt, B.** (1990) 14th International Symposium on Column Liquid Chromatography, Boston, MA. "The use of reversed phase HPLC to characterize norleucine containing variants"
15. Zoller, M.J., Presta, L., Paoni, N., **Keyt, B.**, Botstein, D., Jones, A., Wurm, F., and Bennett, W.F. (1990) *FASEB J.* **4**, number 7, A1695 "Mutational analysis and protein modeling of human tissue plasminogen activator (tPA)"
16. Bennett, W.F., Paoni, N.F., Sethy, I., Kerr, E., Pena, L., **Keyt, B.**, Nguyen, H., Baldini, L., Jones, A., Meunier, A., Wurm, F., Johnson, A., Zoller, M.J., Higgins, D. J., and Botstein, D. (1990) *Fibrinolysis* **4**, suppl.3, 14 "Mapping the Functional Determinants of Tissue Plasminogen Activator"
17. **Keyt, B.**, Nguyen, H., Baldini, L., Gill, J., Brady, K., and Zoller, M. (1990) *Fibrinolysis* **4**, suppl.3, 164 "Identification of determinants on tissue plasminogen activator which mediate active site reactivity, lysine binding and fibrin binding"
18. Refino, C.J., Paoni, N.F., **Keyt, B.A.**, Pater, C., Badillo, J., Bennett, W.F. (1992) Zimmerman Conference: Progress in Vascular Biology, Hemostasis and Thrombosis, La Jolla, CA. "The Properties of Increased Fibrin Specificity, Increased Activity on Platelet Rich Clots and Decreased Plasma Clearance can be combined in a single Human Tissue Plasminogen Activator Variant which exhibits Increased Potency and Limited Systemic Activation In Vivo."
19. Refino, C.J., Paoni, N.F., **Keyt, B.A.**, Pater, C., Badillo, J., Bennett, W.F. (1992) Winter Gordon Conference on Thrombolysis at Ventura, CA. "The Properties of Increased Fibrin Specificity, Increased Activity on Platelet Rich Clots and Decreased Plasma Clearance can be combined in a single Human Tissue Plasminogen Activator Variant which exhibits Increased Potency and Limited Systemic Activation In Vivo."
20. **Keyt, B.**, Baldini, L.T., Brady, K., Meunier, A., Spellman, M. and Bennett, W.F. (1992) *Fibrinolysis* **6**, supplement "Slowly Clearing Mutants of Tissue Plasminogen Activator with Conserved Fibrinolytic Activity."

32. **Keyt, B.**, Berleau, L., Nguyen, H., Refino, C., Paoni, N. and Bennett, W., (1993) *Thromb. Haemostas.* **69**, (6) 992, "Subtle Alterations of Tissue Plasminogen Activator Yield Reduced Plasma Clearance with Conserved Fibrinolytic Activity: A Novel Fibrin Specific tPA Variant with Six fold Increased Potency."
33. Berleau, L.T., Nguyen, H.V., Modi, N., Bennett, W.F. and **Keyt, B.A.**, (1993) Pharmacology '93: 44th Annual ASPET Meeting in San Francisco, "Effects of Glycosylation and Inhibitor Resistance on tPA Pharmacodynamics."
34. Modi, N., Berleau, L.T., Ruppell, J., Eppler, S., Bennett, W. and **Keyt, B.** (1993) American Association of Pharmaceutical Scientists in Orlando, FL. "Pharmacokinetics of Tissue Plasminogen Activator (t-PA) and t-PA Variants in Rabbits."
35. Benedict, C. R., Refino, C., **Keyt, B.**, Paoni, N., Todd, J., Thomas, G. R., and Bennett, W. (1994) American College of Cardiology, 43rd Annual Scientific Session "A New Variant of Tissue Plasminogen Activator with Enhanced Efficacy and Lower Incidence of Bleeding When Compared to Recombinant Human Tissue Plasminogen Activator (Activase)."
36. Alibhai, M., Chow, A. M., Nguyen, H. V., **Keyt, B. A.**, Bennett, W. F., and Paoni, N. F., (1994) Keystone Symposium on Structural and Molecular Biology of Protease Function and Inhibition, "Engineering Tissue-type Plasminogen Activator to be Resistant to Plasma Protease Inhibitors."

Invited Lectures:

Keyt, B. Satellite Workshop on Protein Sequencing at the 6th International Conference on "Methods in Protein Sequence Analysis" in Seattle, WA., August 17-21, 1986

Keyt, B. at the Fourth Annual Ottawa-Carleton Graduate Student Symposium on Perspectives in Biotechnology, University of Ottawa, Ottawa, Canada, February 14, 1987 "Characterization of Natural and Recombinant Human Factor VIIIc"

Keyt, B., Symposium on the "Application of Analytical Chemistry to Protein Characterization", October 5, 1988 at the 27th Annual Eastern Analytical Symposium in New York, NY. "Reversed Phase HPLC Analysis of Recombinant Tissue Plasminogen Activator"

Keyt, B. Tutorial on the Analysis and Purification of Biological Molecules by HPLC at FASEB 72nd Annual Meeting, Las Vegas, NV., May 1-5, 1988 "Role of HPLC in Analysis of Recombinant DNA-Derived Proteins"

Keyt, B., Symposium on the "Purification and Chromatographic Characterization of Proteins", October 6, 1988 at Yale Medical School, New Haven, CT. "Reversed Phase HPLC Analysis of Recombinant Tissue Plasminogen Activator (r-tPA)"

Keyt, B., An International Conference: Advances in the Treatment of Myocardial Infarction, April 11-12, 1991 in London, U.K. "Functional Determinants of Tissue-type Plasminogen Activator"

Keyt, B., Symposium on Biopharmaceutical Half-life Extension, June 18-19, 1992 in New Orleans, LA. "Engineering Longer Half-life Forms of Tissue Plasminogen Activator"

21. **Keyt, B.A.**, Berleau, L.T., Nguyen, H.V., and Bennett, W.F., (1992) *Fibrinolysis* 6, supplement "Radioiodination of the Active Site of Tissue Plasminogen Activator for Ligand Binding Studies"
22. Nguyen, H.V., Refino, C., Pena, L., Brady, K., Badillo, J., Pater, C., Bennett, W. and **Keyt, B.** (1992) *Fibrinolysis* 6, supplement "PAI-1 Resistant Mutants of Tissue-type Plasminogen Activator are Rapidly Cleared in vivo."
23. Refino, C.J., Paoni, N.F., **Keyt, B.A.**, Pater, C.S., Badillo, J.M., Chow, A., Pena, L.C., Nguyen, H.V., Baldini, L.T. and Bennett, W.F. (1992) *Fibrinolysis* 6, supplement "Engineering a Human Tissue Plasminogen Activator that combines Decreased Plasma Clearance and Greater Fibrin Specificity results in a Variant with Increased Potency and Limited Systemic Activation in vivo when administered as a Bolus."
24. Hancock, W., **Keyt, B.**, Guzzetta, A., Bennett, W.F. (1992) HPLC '92 in Baltimore, MD "The Analysis of rtPA by LC/Electrospray Mass Spectrometry and Matrix Assisted Laser Desorption"
25. Modi, N.B., Berleau, L.T., Uchizono, J. A., Thomsen, K., Nixon, A., Badillo, J.M., Refino, C.J., and **Keyt, B.A.** (1993) Fifth European Congress of Biopharmaceutics and Pharmacokinetics in Brussels, "Pharmacokinetics and Pharmacodynamics of Activase® t-PA: Comparison of Intravenous Bolus and Infusion Regimens in Rabbits"
26. Sehl, L., Nguyen, H.V., Arcila, P., **Keyt, B.A.** and Bennett, W.F., (1993) *Thromb. Haemostas.* 69, (6) 577, "A Comparison of the Properties of C83A and C84A Mutants of Tissue-type Plasminogen Activator (t-PA): Evidence Indicating That C83 Is Unpaired"
27. Chow, A.M., Pena, L.C., **Keyt, B.A.**, Zoller, M.J., Bennett, W.F. and Paoni, N.F., (1993) *Thromb. Haemostas.* 69, (6) 762, "Increasing the Fibrin Specificity of Tissue Plasminogen Activator"
28. Nguyen, H., Arcila, P., Clark, K., Fendly, B., Bennett, W. and **Keyt, B.**, (1993) *Thromb. Haemostas.* 69, (6) 826, "Epitope Mapping of Monoclonal Antibodies Against Tissue-Type Plasminogen Activator using Alanine Scan Variants"
29. Berleau, L., Nguyen, H., Modi, N., Bennett, W. and **Keyt, B.**, (1993) *Thromb. Haemostas.* 69, (6) 840, "Pharmacokinetics and Pharmacodynamics of tPA Variants in Rabbits"
30. Collen, D., Stassen, J. M., Refino, C. J., Paoni, N. F., **Keyt, B. A.**, Roskams, T., Jang, I. K., Lijnen, H. R., Gold, H. K., and Bennett, W. F., (1993) *Thromb. Haemostas.* 69, (6) 841, "Comparative Thrombolytic Properties of Recombinant Tissue-Type Plasminogen Activator (rt-PA) and of Two Plasminogen Activator Inhibitor -1 (PAI-1) Resistant Glycosylation Variants in a Combined Arterial and Venous Thrombosis Model in the Dog."
31. Refino C.J., **Keyt, B.A.**, Paoni, N.F., Badillo, J.M., Pater, C.J., Van Peborgh, J., Pena, L., Berleau, L.T., Nguyen, H.V. and Bennett, W.F., (1993) *Thromb. Haemostas.* 69, (6) 841, "A Variant of Tissue Plasminogen Activator (T103N, N117Q, KHR296-299AAAA) with a Decreased Plasma Clearance Rate, is Substantially More Potent than Activase® rt-PA in a Rabbit Thrombolysis Model"

Patents Granted:

In Australia, New Zealand and Spain:

Anderson, Stephen and Keyt, Bruce A.

Serial No. 07/196909

Docket No. 488

Filed: May 20, 1988

Granted: Spain (Mar 16, 1990) as Patent 8901686

New Zealand (Jul 25, 1991) as Patent 229081

Australia (Sep 28, 1992) as Patent 624158

For: Variants of Plasminogen Activators and Processes for their Production

In the United States:

Bennett, W. F., Keyt, Bruce A. and Zoller, Mark, J.

Patent No. 5246850

Serial No. 07/560,515 and 07/912040

Docket No. P650 and P650C1

Filed: July 31, 1990

Granted: Sept. 21, 1993

For: Tissue Plasminogen Activator Variants with Decreased Clearance

Patent Applications Pending:

Anderson, Stephen and Keyt, Bruce A.

Serial No. 07/196909, 07/480691, 07/824740, 08/060290, 08/035427, 08/036014

Docket No. 488, 488P1, 488P1C1, 488P1C1D1, 488P1C2, 488P1C3

Filed: May 20, 1988

For: Variants of Plasminogen Activators and Processes for their Production

Anderson, Stephen and Keyt, Bruce A.

International Application Number: PCT-US89-01947

International Filing Date: May 4, 1989

International Publication Number: WO 89-11531

International Publication Date: Nov. 13, 1989

For: Variants of Plasminogen Activators and Processes for their Production

Anderson, S., Keyt, B. A., Brady, K., Presta, L. G.

Serial No. 07/480,691

Docket No. 488P1

Filed: February 15, 1990

For: Variants of Plasminogen Activators and Processes for their Production

Bennett, William F. and Keyt, Bruce A.

Serial No. 07/808121

Docket No. 744

Filed: December 16, 1991

For: Tissue Plasminogen Activator Substitution Variants with Improved Fibrin-Specificity

Anderson, S., Keyt, B., Brady, K., Presta, L. G.
Serial No. 07/824,740
Docket No. 488P1C1
Filed: January 21, 1992
For: Variants of Plasminogen Activators and Processes for their Production

Bennett, W. F., Keyt, B. A. and Paoni, N. P.
Serial No.
Docket No. 757
Filed: June 3, 1992
For: Tissue Plasminogen Activator Glycosylation Variants with Increased In Vivo
Fibrinolytic Potency